



Prenatal diagnosis following preimplantation genetic testing (PGT): recommendations of the Italian Society of Human Genetics (SIGU)

Francesca Romana Grati¹ · Antonio Capalbo^{2,3} · Ilaria Gabbiato⁴ · Paola Battaglia⁵ · Maria Carla Pittalis⁵ · Domenico Bizzoco⁶ · Laura Cardarelli⁷ · Valentina Gatta² · Fortunato Lonardo⁸ · Antonio Novelli⁹ · Laura Bernardini¹⁰ · Daniela Zuccarello⁴ on behalf of on behalf of the Italian Society of Human Genetics (SIGU)

Received: 16 September 2024 / Accepted: 12 December 2024 / Published online: 28 January 2025
© The Author(s) 2024

Abstract

This document aims to provide good practice recommendations in order to support maternal-foetal medicine specialists, clinical geneticists and clinical laboratory geneticists in the management of pregnancies obtained after the transfer of an embryo tested with preimplantation genetic testing (PGT). It was drafted by geneticists expert in preimplantation genetics and prenatal genetic diagnosis belonging to the “Working Group in Cytogenomics, Prenatal and Reproductive Genetics” of the “Italian Society of Human Genetics” (SIGU). In particular, the paper addresses the diagnostic algorithm to be applied in prenatal follow-up depending on the type of PGT performed, the results obtained and the related diagnostic value based on the most recent literature data and Italian and international recommendations.

Keywords Embryo · PGT-M · PGT-SR · PGT-A · Mosaicism · Prenatal diagnosis

Introduction

PGT is the earliest genetic investigation performed on products of conception, as it allows for verification of possible hereditary genetic diseases and chromosomal alterations in embryos obtained from in vitro fertilisation, before their transfer to the uterus [1].

PGT is a test that can inform couples about the health status of their embryos before the onset of pregnancy, in order to minimise the risks of foetal chromosomal and genetic syndromes resulting in spontaneous/therapeutic abortion [2].

Recent progress in understanding the human genome and in molecular diagnostic methods has led to expanded knowledge and awareness of the mechanisms underlying an ever-increasing number of hereditary diseases, attributable to genetic alterations.

Genetic/familial risk assessment is particularly important for couples undergoing assisted reproductive technologies (ART), in order to make informed reproductive choices.

PGT is performed as part of a II level ART protocol, which includes oocyte retrieval and semen collection, in vitro fertilisation and culture of the embryos, and analysis at the blastocyst stage (5–7th day of in vitro development) by biopsy of 5–10 cells of the trophectoderm [3], which will give origin to the placenta and foetal annexes [4, 5].

Francesca Romana Grati and Antonio Capalbo contributed equally to the manuscript.

✉ Laura Bernardini
l.bernardini@css-mendel.it

¹ Reproductive Precision Medicine Unit, Menarini Silicon Biosystems, Castel Maggiore, Bologna, Italy

² Unità Di Genetica Medica, CAST, Università “G. d’Annunzio” Chieti-Pescara, Chieti, Italy

³ Reproductive Genetics, Juno Genetics, Rome, Italy

⁴ Medical Genetics & Genomics Unit, AULSS8 Berica, Vicenza, Italy

⁵ Medical Genetics Unit, IRCCS Azienda Ospedaliero-Universitaria Di Bologna, Bologna, Italy

⁶ Laboratorio Di Genetica MEDIGENE, Rome, Italy

⁷ RDI - Gruppo Cerba HealthCare Italia, Limena, Padua, Italy

⁸ U.O.S.D. di Genetica Medica, A.O.R.N San Pio, Benevento, Italy

⁹ U.O.C. Laboratorio Di Genetica Medica, Ospedale Bambino Gesù, IRCCS, Rome, Italy

¹⁰ Medical Genetics Division, San Giovanni Rotondo, Casa Sollievo della Sofferenza Foundation, Foggia, FG, Italy

This technique can be considered a proxy for a very early direct preparation of chorionic villi, specifically investigating the progenitor cells of the outer layer of the placenta, known as cytotrophoblasts [6]. The obtained sample can be used to investigate aneuploidies or the possible transmission of genomic imbalances and monogenic diseases from one or both carrier members of the couple.

It is particularly important that the PGT centres follow the Italian and international recommendations to guarantee the validity of the test result [7, 8].

Indications to PGT

PGT can be performed because of different indications; it can be divided into three different types: PGT for monogenic diseases (PGT-M), PGT for chromosomal structural rearrangements (PGT-SR) and PGT for aneuploidies (PGT-A) [9].

In detail, PGT-M can be performed for autosomal dominant, autosomal recessive, X-linked diseases and in the case of HLA typing [10]. PGT-SR is indicated in cases where one or both partners in the couple have a structural aberration affecting one or more chromosomes (mainly translocations, but also inversions, deletions and duplications). Finally, PGT-A analyses the entire set of chromosomes to investigate de novo numerical alterations of whole chromosomes (aneuploidies) or partial imbalances (aneusomies). The main indications for PGT-A are advanced maternal age, recurrent miscarriages, repeated implantation failures and chromosomal mosaicism in one or both components of the couple [11].

Preimplantation genetic testing for monogenic diseases: PGT-M

PGT-M is indicated for couples carrying single gene variants leading to autosomal dominant, recessive or X-linked diseases.

In order to assess the feasibility of PGT-M during pre-PGT genetic counselling, it is necessary to know the molecular basis of the familial disease [12]. Before the embryo analysis, a preclinical set-up needs to be performed to verify the feasibility of the direct testing of the specific pathogenic variant and the informativity of polymorphic markers to be used in the indirect analysis. Therefore, after the collection of DNA samples from the couple and available relatives, the customised test is developed and validated by identifying the primers for amplification of the specific pathogenic variant and the informative molecular markers (short tandem repeats ‘STR’ or single nucleotide polymorphisms ‘SNP’) flanking the variant, to identify the segregating at-risk haplotype.

Identifying informative markers linked to the specific variant is essential to minimise the likelihood of false negative results due to allele dropout (ADO), which can be a confounding factor when using DNA extracted from only a few cells [13]. Indeed, an indirect analysis by examining the at-risk haplotype, from which the possible presence/absence of the variant itself can be inferred and ultimately confirmed, is performed in addition to the direct investigation of the gene variant. The haplotype analysis can be directly carried out on the embryos at the PGT-M analysis stage in the case that familial samples are not available.

Once the feasibility of the analysis has been ascertained, after the preclinical set-up, the ART cycle can be started to obtain a sufficient number of embryos for the analysis. PGT-M is then performed on the DNA extracted from the blastocysts, and the results are discussed with the couple to plan the in utero transfer of the non-affected embryos.

The main analytical techniques used in PGT are next-generation sequencing (NGS), chromosomal microarray analysis (CMA), mainly SNP-arrays, Sanger sequencing and quantitative PCR (qPCR). These methods provide high qualitative and quantitative accuracy (> 99%) allowing high success in inferring embryo genotype [14].

Preimplantation genetic testing for structural rearrangements: PGT-SR

PGT-SR is indicated in couples where one (or both) partner carries a structural chromosomal abnormality. The most common structural abnormalities are translocations, followed by inversions, deletions and duplications. Individuals carrying balanced structural rearrangements are usually healthy but have an increased risk of producing unbalanced zygotes.

As with PGT-M, the feasibility of PGT-SR is assessed during pre-PGT genetic counselling. This is based on a laboratory report describing the size and breakpoints of the rearrangement. If these data meet criteria related to the resolution of the platform used for PGT-SR, the couple can start the ART cycle. This test does not require any pre-clinical preparation. A familial copy number variation (CNV) with a size below the resolution limit of the platform used by the PGT laboratory represents an indication similar to those discussed for PGT-M. Therefore, also in such a case, a pre-clinical set-up can be attempted for the identification of the at-risk haplotype [15].

Since PGT-SR is generally performed by genome-wide technologies (currently mainly NGS), besides the expected unbalanced segregation of the family rearrangement, the embryos are simultaneously examined for the presence of de novo genome-wide imbalances (aneuploidies and aneusomies). Typically, depending on the genomic region

under investigation, the average resolution of this analysis is 5–7 Mb [16]. In selected cases, fluorescent in situ hybridisation (FISH) analysis is used to visualise only the specific chromosomal regions involved in the rearrangement [2].

Preimplantation genetic testing for chromosomal aneuploidies: PGT-A

PGT-A allows the identification of embryos with de novo aneuploidies or partial imbalances with an approximate resolution of 5–7 Mb, which varies depending on the analytical method [17]. It is aimed at identifying euploid embryos, i.e. those with the highest implantation and full-term pregnancy rates. The main indications for PGT-A are as follows: advanced maternal age (≥ 35 years), which is associated with an increased risk for foetal aneuploidies, repeated implantation failures following embryo transfer, termination of pregnancy and/or multiple spontaneous abortions (idiopathic or chromosomal) and chromosomal mosaicism in one or both members of the couple [18]. Meta-analysis and randomised studies indicate that the main advantages of this analysis are the reduction of time needed to reach a full-term pregnancy and the reduction of chromosomal miscarriages or pregnancy terminations rates [19, 20]. PGT-A is also recommended on embryos with an indication for PGT-M, regardless of the risk associated with maternal age, in order to evaluate their health status and maximise the probability of obtaining a full-term pregnancy.

PGT-A is a genetic test with high specificity (i.e. low false positive rate, $< 5\%$) and sensitivity (i.e. low false negative rate, $< 1\%$). However, the finding of two or more different chromosomal sets (putative mosaicism), which is estimated to occur with an incidence of approximately 4–5% at the blastocyst stage [21], can give rise to divergent analytical data, due to possible karyotype discordance between TE and inner cell mass (ICM), from which the foetus will originate.

Post-PGT pregnancy follow-up

In pregnancies achieved through the transfer of an embryo subjected to preimplantation genetic testing, it is advisable for the couple to undergo prenatal genetic counselling, during which the possibility of conducting prenatal genetic investigations will be offered and discussed as follows:

- To estimate the residual risk after PGT as it allows an informed decision on whether or not to undergo invasive prenatal diagnosis; this varies greatly depending on the type of PGT performed, the assay technique used and the pathology detected;

- PGT analysis of small number of cells having the same embryological origin as the external epithelial layer of the placenta, which is derived from the trophoectoderm. Amniocentesis, compared to chorionic villus sampling, allows access to embryo tissues with a closer embryological proximity to the foetus. However, should the couple decide to undergo invasive prenatal diagnosis to obtain a better definition of the foetal genetic status and reduce the residual risk, it is appropriate to discuss the best option between chorionic villus sampling (CVS) and amniocentesis. In fact, there are cases in which CVS can still be proposed, depending on the diagnostic indication and/or the PGT results (see below);
- PGT is aimed at investigating specific genetic conditions, therefore standard ultrasound pregnancy monitoring (e.g. three recommended foetal ultrasounds: first trimester with measurement of nuchal translucency; second trimester for foetal morphology; third trimester for foetal growth) and routine antenatal evaluations [22] must be maintained as a standard of care.

In cases of a high residual risk after PGT, the clinical geneticist may recommend additional foetal investigations or invasive diagnostic tests on a case-by-case basis.

There is still conflicting evidence about a possible less favourable placental outcome in pregnancies with an embryo transferred after a second biopsy (with thawing and refreezing), performed because of an initial inconclusive PGT result. Nonetheless, collected data indicates that the neonatal outcome is unchanged. Therefore, in these cases, it is currently suggested to check the placental functionality following the standard monitoring in the third trimester of pregnancy [23, 24].

Prenatal follow-up after PGT-M

There is not an international consensus on the management of pregnancies achieved after PGT-M. However, in addition to the general considerations listed above, specific guidance can be given for selected situations.

- If the set-up has determined that the expected sensitivity is lower than the standard or if the final PGT-M report shows an event that might have caused a decreased sensitivity or accuracy compared with what was assumed from the set-up stage, it is necessary to estimate the residual risk and inform the couple about the possibility of confirming the PGT-M result through invasive prenatal diagnosis. If appropriate, a targeted ultrasound follow-up should be planned.

In general, as described in the literature, when the PGT significantly lowers the residual risk ($\leq 1\%$), the percentage of couples opting for invasive prenatal diagnosis as a diagnostic confirmation is small (around 10%). In fact, since a pregnancy obtained through PGT is considered particularly “valuable,” the majority of couples refuse the invasive prenatal test because a 99% risk reduction is considered acceptable [25].

- If the couple opts for an invasive confirmatory testing, chorionic villus sampling may be considered, as the pathogenic variant is meiotically transmitted and thus expected to be homogeneously present in all foetal and extra-foetal tissues.

Finally, when PGT-M was not combined with PGT-A, follow-up management is recommended based on the maternal age-associated risks (Fig. 1).

Prenatal follow-up after PGT-SR

There is not an international consensus on the management of pregnancies achieved by PGT-SR. However, in addition to the general considerations listed above, specific guidance can be given for selected situations.

- When PGT-SR is performed in a couple with a structural abnormality involving imprinted chromosomes (6, 7, 11, 14, 15, 20), it is necessary to ensure that the technique used in PGT has excluded an eventual uniparental disomy (UPD). If not, it is important to discuss with the couple

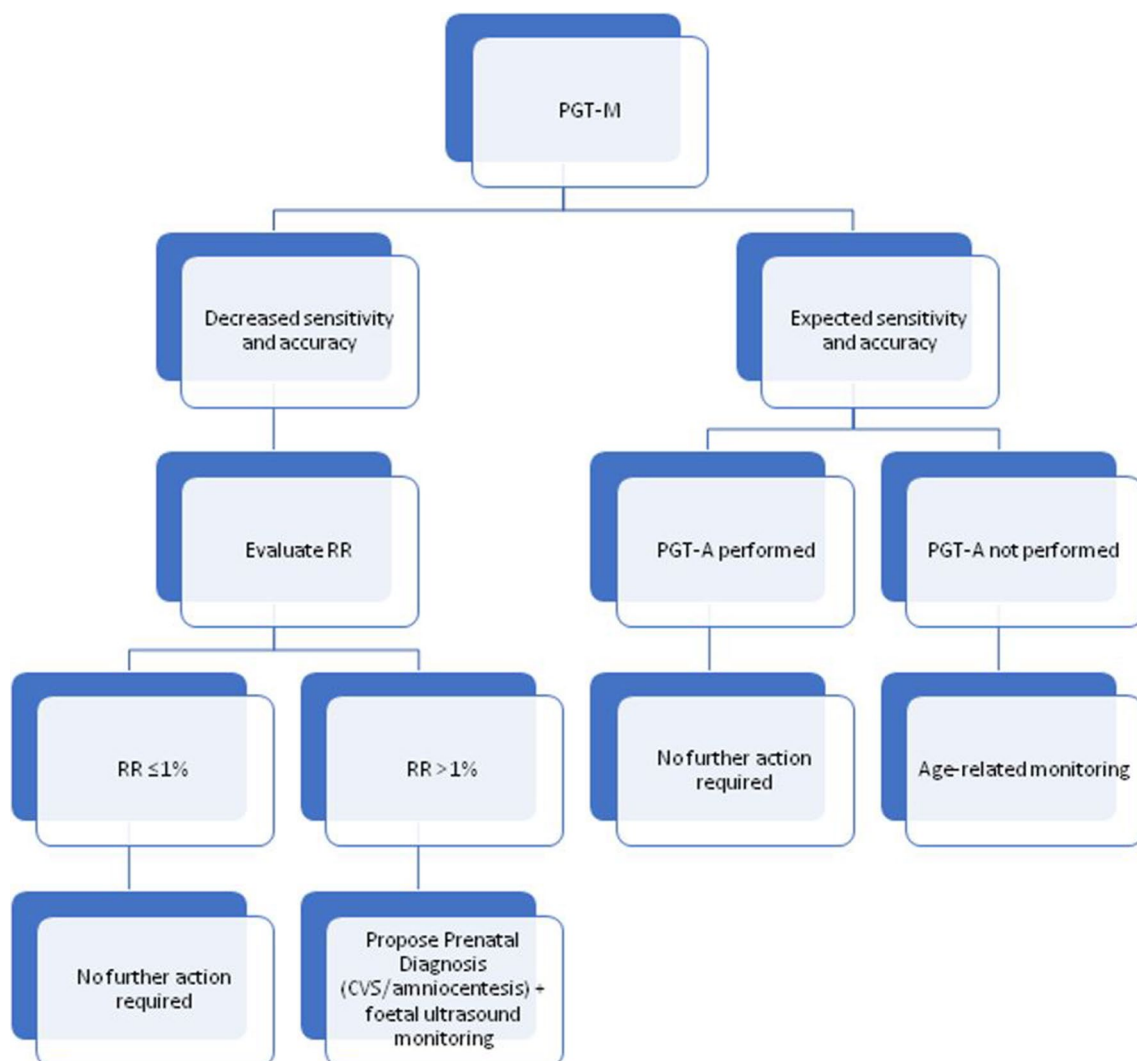


Fig. 1 Prenatal follow-up after PGT-M. PGT-M, preimplantation genetic testing for monogenic diseases; RR, residual risk; CVS, chorionic villus sampling; UPD, uniparental disomy

the residual risk of UPD for the specific chromosome involved (usually not exceeding 1%) [26] and its potential clinical consequences, proposing the execution of invasive prenatal diagnosis through chorionic villus sampling (CVS) or amniocentesis. Chorionic villus sampling or amniocentesis are both acceptable, as the eventual UPD in these cases arises as a consequence of a meiotic mechanism, therefore expected to be present homogeneously in the embryo.

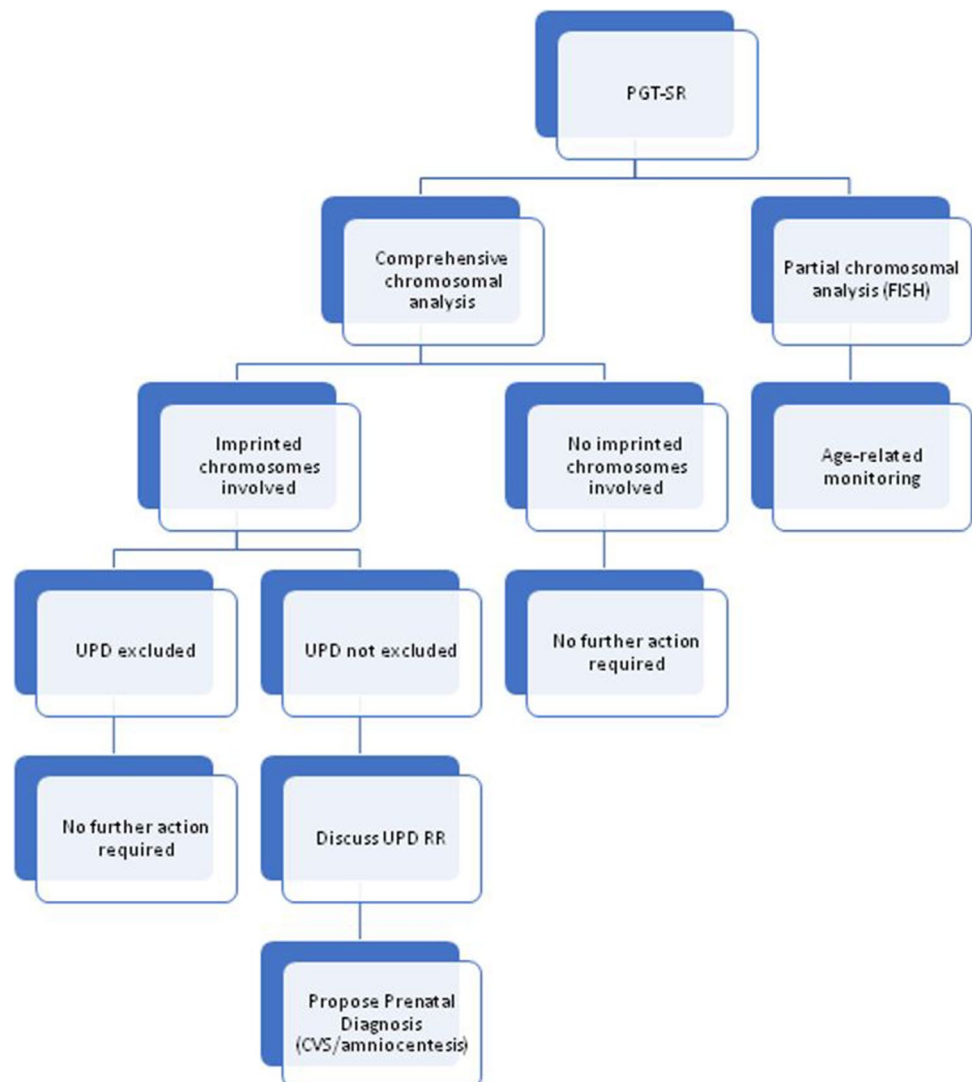
- If PGT-SR was not carried out with a genome-wide assay extended to all chromosomes and not only those involved in familial structural rearrangements (e.g. FISH), the monitoring of the pregnancy based on the maternal age-associated risks is recommended [27, 28] (Fig. 2)

Prenatal follow-up after PGT-A

Prenatal follow-up after euploid embryo transfer at PGT-A

The main goal of PGT-A is to reveal the presence of uniform aneuploidies of likely meiotic origin causative of negative pregnancy outcomes in IVF treatment cycles. Couples should be made aware that the risk of foetal aneuploidy after a euploid PGT-A result is lower than the risk of the general prenatal population [29]; therefore, a standard pregnancy follow-up monitoring is usually recommended, also for women of advanced reproductive age. However, it is appropriate to discuss with the couples the residual risk for foetal aneuploidies after a euploid PGT-A result, so that they can evaluate the opportunity to perform an invasive prenatal diagnosis. In fact, although very rare, there

Fig. 2 Prenatal follow-up after PGT-SR. PGT-SR, preimplantation genetic testing for chromosomal structural rearrangements; RR, residual risk; CVS, chorionic villus sampling; UPD, uniparental disomy



might be a diagnostic discordance due to the presence of a chromosomal mosaicism at the blastocyst stage, which is a well-known biological phenomenon related to the analysis of the placental precursors with an estimated frequency of approximately 4–5% in blastocyst stage embryos [21]. In this case, amniocentesis is recommended, because it analyses foetal cells, thus minimising the risk of detecting *de novo* aneuploidy/aneusomy confined to extraembryonic tissues.

It is not appropriate to perform a first-trimester combined test (FCT) for trisomy 13, 18, and 21, combining serum analytes with nuchal translucency thickness, as it shows a lower performance (sensitivity and specificity) than PGT-A [30]. Furthermore, the algorithm used for risk calculation in such pregnancies cannot take into account that the risk related to maternal age is reduced after PGT-A.

Likewise, NIPT test (non-invasive prenatal test) is not indicated, as circulating cell-free foetal DNA has the same embryological origin as the tissue investigated in PGT-A [31].

Finally, it is important to inform the couple that there is still a residual risk for pathogenic CNVs smaller than 5–7 Mb in size, below the resolution of PGT-A. They have a frequency of 1/100–200 in the general obstetric population and are responsible for a significant burden of perinatal morbidity and mortality [32, 33].

Prenatal follow-up following the transfer of a mosaic embryo at PGT-A

PGT-A can reveal intermediate chromosomal copy number profiles, defined as a value between the euploidy (two copies) and aneuploidies (one for monosomy and three for trisomy) thresholds, a condition commonly referred to as presumed or putative chromosomal mosaicism. The diagnosis of chromosomal mosaicism in PGT-A presents significant analytical problems, as the test is performed on samples with very low cell numbers and DNA input. This is associated with a high inter- and intra-laboratory analytical variability inherently caused by the several amplification steps that characterise all PGT-A methodologies. As a consequence, it is impossible to define whether a chromosomal profile of intermediate value is due to analytical variability or to a true biological finding. Nevertheless, there are extensive clinical experiences that allow to conclude that embryos with presumed PGT-A mosaicism have a probability of full-term pregnancy and obstetric/neonatal risks comparable to uniformly euploid embryos. Therefore, this finding supports the practice whereby these embryos can/must equally be considered suitable for transfer in IVF cycles, although it should be noted that the question regarding the risk of congenital anomalies and possible long-term effects still remains unsolved [34–38]. Although it is technically unfeasible to define the percentage of mosaicism within a

sample with an undefined cell quantity, intermediate copy number profiles can commonly be expressed as a percentage of deviation from the expected (two copies) using various bioinformatic algorithms. It is a common practice for some laboratories to also report the percentage of presumed mosaicism in TE biopsies, either expressed as exact percentage values or categorised as low/high grade. To evaluate the reproductive competence and clinical value of intermediate copy number in TE biopsies, two types of studies have been performed: retrospective, where mosaicism is outlined in the reports prior to transfer, or prospective, where this is not reported but evaluated only following the embryo transfer. The limitation of retrospective studies consists in the fact that embryos defined as putative mosaics are transferred as the last option and therefore in patients with previous failures and poor prognosis. It effectively introduces a strong selection bias which undermines the correct evaluation of the reproductive potential of these embryos. In blinded prospective studies, this bias is not introduced, and a more accurate comparison of clinical outcomes is obtained [39]. In any case, all published studies have highlighted a high reproductive potential of embryos with presumed mosaicism which, in blind prospective studies, is even equivalent to that of euploid embryos [37, 40, 41]. In consideration of the evidence obtained to date, a recent publication by the European Society of Human Reproduction [42] has outlined guidelines regarding the finding of putative mosaicisms in preimplantation embryos, which are summarised here:

- The detection of presumed chromosomal mosaicism in the few cells of the biopsy may not reflect the constitution of the entire embryo, nor can it predict its viability.
- Laboratories that perform PGT can decide whether or not to report the finding of presumed mosaicism in the final PGT-A report. This decision must be communicated to the couple, during the pre-test counselling, together with the parameters used to define an embryo suggestive of mosaic and what threshold the laboratory has established to define a high- or low-grade mosaic. The European Recommendations suggest that a threshold lower or higher than 50% should be used to define the degree of low- or high-grade mosaicism, respectively.
- Due to the high diagnostic inaccuracy and uncertainty of mosaicism on samples of just a few cells, it is not possible to attribute a diagnostic value to the finding of embryonic mosaicism. It is suggested to use more appropriate terminology, such as pseudo-mosaicism, apparent mosaicism, or presumed mosaicism.
- It is not possible to consider an embryo with apparent low-grade mosaicism following PGT-A as aneuploid.
- It is not recommended to perform a new IVF cycle if the couple still has embryos with presumed low-grade mosaicism available for transfer,

- It is not recommended to discard embryos with a presumed low degree of mosaicism.
- It is not recommended to proceed with a second biopsy of embryos with a low degree of presumed mosaicism to confirm their chromosomal status.
- Given the heterogeneity in the quality metrics adopted by various laboratories, discriminating between a presumed low-grade mosaic and a technical artefact may not always be possible. In these instances, the laboratory may be led to report as “low-grade mosaic” profiles that present suggestive deviations in correspondence of specific chromosomes because they showed low-quality profiles.

For these reasons, according to the European Recommendations, the transfer of a presumed low-grade mosaic embryo is not associated with an increased risk of negative clinical outcomes compared to pregnancies obtained from uniformly euploid embryo transfer, and therefore, it does not represent an indication to recommend invasive prenatal diagnosis. In any case, as with the transfer of euploid embryos, all women who decide to transfer an embryo with presumed low-grade mosaicism should be offered genetic counselling to discuss prenatal follow-up of the pregnancy and the possibility of performing invasive prenatal diagnosis, recommending amniocentesis in such cases. In the event of a transfer of embryos with presumed high-grade mosaicism, it is necessary to discuss during genetic counselling the implications of a pregnancy obtained from this type of embryo and the prenatal follow-up, as well as recommending the performance of invasive prenatal diagnosis. In fact, it is conceivable that the majority of these presumed high-grade mosaic embryos do not implant or undergo spontaneous abortion as a consequence of aneuploidy. However, there is still the possibility that part of them could implant and reach a full-term pregnancy [43]. In this case, possible unfavourable outcomes are the onset of altered imprinting syndromes (when the aneuploidy involves imprinted chromosomes), true foetal mosaicism, the birth of an individual affected by mosaic aneuploidy (e.g. trisomy 8, trisomy 9), or with an abnormal karyotype resulting from the partial correction of aneuploidy [44].

In the case of invasive prenatal diagnosis in pregnancies obtained from the transfer of embryos with presumed chromosomal mosaicism, the analysis must follow the work-up criteria established by the current version of the SIGU “Document of Good Practice for Constitutional Cytogenetic and Cytogenomics Diagnosis” (Table 1).

It is therefore essential that the prenatal genetic laboratory is made aware of PGT-A results upon receipt of the sample in order to conduct the most appropriate laboratory analysis. If a presumed mosaic embryo with aneuploidies or segmental aneusomies involving imprinted chromosomes 6, 7, 11, 14, 15, and 20 has been transferred, it is necessary to integrate the analysis of the foetal karyotype with the exclusion of UPD. These laboratory data, conducted with systematic work-ups for the investigation of potential mosaicism, are necessary to fill knowledge gaps regarding the outcomes of presumed mosaicism identified at PGT-A. Equally important are the prospective collections of clinical outcomes up to at least 2 years of life of the unborn child [38].

If during PGT-A a segmental aneusomy in a presumed mosaic is reported, pre-transfer genetic counselling is recommended to describe the uncertainties of the prenatal management of a possible pregnancy obtained from the transfer of this embryo. It is necessary to explain to the couple:

- that the resolution limit of the method used is approximately 5–7 Mb;
- that segmental aneusomies have an incidence of 5–6% and are mostly of mitotic origin and not related to maternal age [45];
- that the chances of confirmation/persistence of the segmental aneusomy on foetal samples are extremely rare [46];
- that in the case of confirmation of the imbalance, the prediction of its pathogenicity and the consequent impact on the foetal phenotype can be difficult, especially if the imbalance is a duplication, for which size alone is not a reliable prognostic factor;
- that a partial foetal imbalance, especially if mosaic, may not give detectable ultrasound signs and alterations, even at advanced gestational weeks.

Table 1 Recommended workup in prenatal diagnosis of pregnancies obtained by transfer of a mosaic embryo (from: “Document of Good Practice for Constitutional Cytogenetic and Cytogenomics Diagnosis” [47])

Method	Minimal number of metaphases/clones to count	Minimal number of independent cultures
CVS direct preparation	12 metaphases	-
CVS/AF flask method	30 metaphases	2
In situ method	20 clones	3
Foetal blood culture	50 metaphases (100 if a cell with the anomaly under investigation has been found)	2

AF amniotic fluid, CVS chorionic villus sample

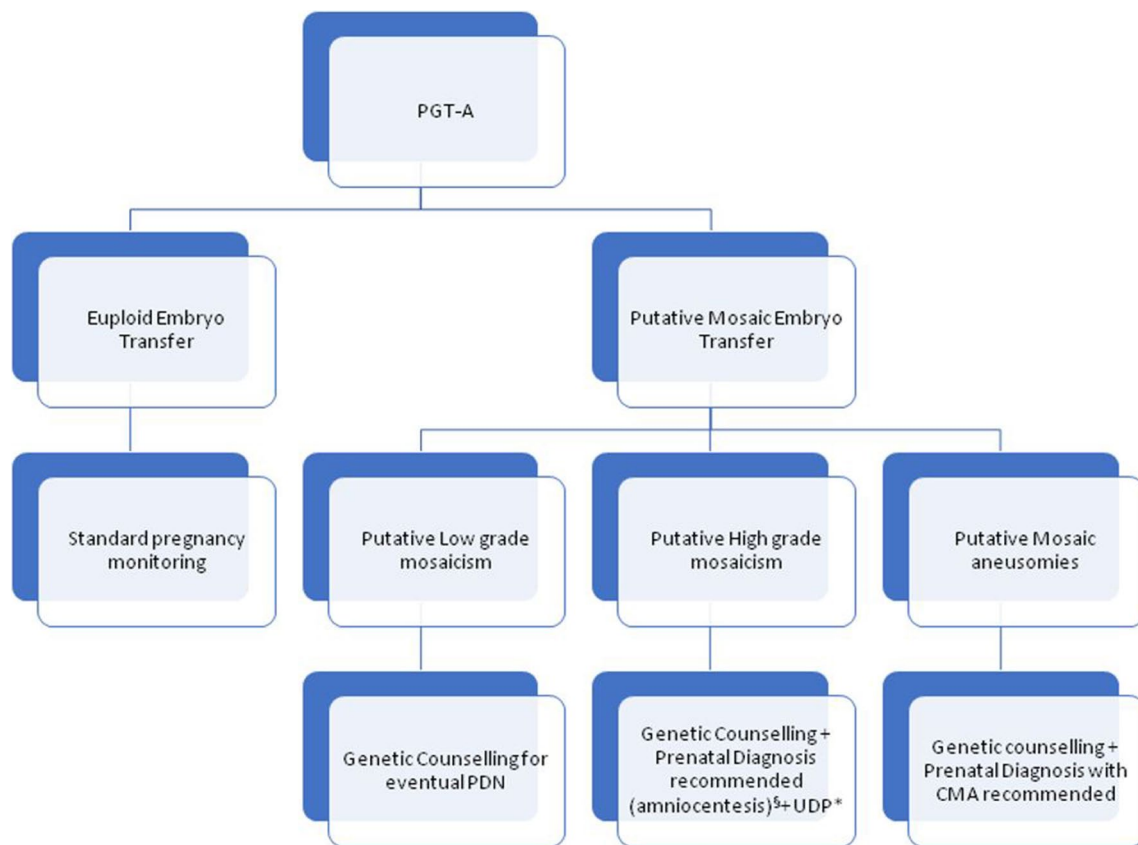


Fig. 3 Prenatal follow-up after PGT-A. PGT-A, preimplantation genetic testing for aneuploidies; PDN, prenatal diagnosis; UDP, uniparental disomy; CMA, chromosomal microarray analysis. §As recommended in Table 1. *In case of involvement of imprinted chromosomes

In case of a clinical pregnancy following transfer of mosaic segmental aneusomy, it is recommended to carry out an invasive prenatal diagnosis which includes CMA [47] and adequate foetal ultrasound monitoring (Fig. 3).

Conclusions

These recommendations, produced by a national scientific society, are the first official document produced by expert geneticists in the field of prenatal diagnosis providing specific guidelines for the management of pregnancies achieved by IVF-PGT techniques. We are confident that it will be a useful tool for medical geneticists and gynaecologists dealing with pregnant women who have undergone PGT, with the aim of rationalising invasive prenatal genetic testing for medically assisted reproduction pregnancies.

Acknowledgements We thank the Italian Society of Human Genetics (SIGU) and European Union for supporting this work and Dr. Alessandro De Luca for his support in revising and editing the final version of the manuscript.

Author contribution A.C., F.R.G., and D.Z. conceptualised this work and with I.G. initiated the drafting of the recommendations. All authors participated to write and revise the original document and approved the final version of this manuscript. L.B., P.B., A.C., F.R.G., I.G., M.C.P., and D.Z. wrote the recommendations in English. L.B. contributed to conceptualise the document and provided the working group supervision.

Declarations

Conflict of interest F.R.G. is currently a full-time employee of Menarini Silicon Biosystems (Castel Maggiore, Bologna, Italy); at the time of document conceptualization, she was an employee of TOMA Advanced Biomedical Assays S.p.A. (ImpactLab) and the coordinator of the SIGU working group of cytogenetics and cytogenomics. L.C. is employee of RDI – Italian Diagnostic Network, Cerba Healthcare Italy Group. The other authors have no conflict of interest to report related with this manuscript.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons

licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. *Curr Opin Obstet Gynecol*. 1999;11(3):307–11. <https://doi.org/10.1097/00001703-199906000-00013>.
- Takeuchi K. Pre-implantation genetic testing: past, present, future. *Reprod Med Biol*. 2020;20(1):27–40. <https://doi.org/10.1002/rmb2.12352>.
- Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril*. 2013;100(3):624–30. <https://doi.org/10.1016/j.fertnstert.2013.04.039>.
- Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Canipari R, Rienzi L. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. *Biomed Res Int*. 2016;2016:7193075. <https://doi.org/10.1155/2016/7193075>.
- Maggiulli R, Giancani A, Cimadomo D, Ubaldi FM, Rienzi L. Human blastocyst biopsy and vitrification. *J Vis Exp*. 2019;26:(149). <https://doi.org/10.3791/59625>.
- ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group; Kokkali G, Cotichio G, Bronet F, Celebi C, Cimadomo D, Goossens V, Liss J, Nunes S, Sfountouris I, Vermeulen N, Zakharova E, De Rycke M. ESHRE PGT Consortium and SIG Embryology good practice recommendations for polar body and embryo biopsy for PGT. *Hum Reprod Open*. 2020;2020(3):hoaa020. <https://doi.org/10.1093/hropen/hoaa020>.
- ESHRE PGT Consortium Steering Committee; Carvalho F, Coonen E, Goossens V, Kokkali G, Rubio C, Meijer-Hoogveen M, Moutou C, Vermeulen N, De Rycke M. ESHRE PGT Consortium good practice recommendations for the organisation of PGT. *Hum Reprod Open*. 2020;2020(3):hoaa021. <https://doi.org/10.1093/hropen/hoaa021>.
- Raccomandazioni SIERR - SIGU di buona pratica per la predisposizione e l'organizzazione di laboratori di embriologia clinica e genetica medica che eseguono test genetici preimpianto, 2024. https://sigu.net/wp-content/uploads/2023/11/documento-SIGU-SIERR_finale_22012024-1.pdf.
- Diagnosi Genetica Preimpianto - PGT Raccomandazioni SIGU 2017 per la pratica clinica. https://sigu.net/wp-content/uploads/2020/11/2043-2017_08_09_Raccomandazioni-PGT2017-ApprovatoCDSIGU.pdf.
- ESHRE PGT-M Working Group; Carvalho F, Moutou C, Dimitriadou E, Dreesen J, Giménez C, Goossens V, Kakourou G, Vermeulen N, Zuccarello D, De Rycke M. ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders. *Hum Reprod Open*. 2020;2020(3):hoaa018. <https://doi.org/10.1093/hropen/hoaa018>.
- ESHRE PGT-SR/PGT-A Working Group; Coonen E, Rubio C, Christopikou D, Dimitriadou E, Gontar J, Goossens V, Maurer M, Spinella F, Vermeulen N, De Rycke M. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. *Hum Reprod Open*. 2020;2020(3):hoaa017. <https://doi.org/10.1093/hropen/hoaa017>.
- De Rycke M, Berckmoes V. Preimplantation genetic testing for monogenic disorders. *Genes (Basel)*. 2020;11(8):871. <https://doi.org/10.3390/genes11080871>.
- Volozonoka L, Perminov D, Korņejeva L, Alksēre B, Novikova N, Pīmane EJ, Blumberga A, Kempa I, Miskova A, Gailīte L, Fodina V. Performance comparison of two whole genome amplification techniques in frame of multifactor preimplantation genetic testing. *J Assist Reprod Genet*. 2018;35(8):1457–72. <https://doi.org/10.1007/s10815-018-1187-4>.
- Huang P, Lan Y, Zhou H, Lin L, Shu J, Wang C, Zhao X, Liang L, He S, Mou J, Zhang X, Qiu Q, Wei H. Comprehensive application of multiple molecular diagnostic techniques in pre-implantation genetic testing for monogenic. *Mol Genet Genomic Med*. 2024;12(1):e2293. <https://doi.org/10.1002/mgg3.2293>.
- Hu X, Wang W, Luo K, Dai J, Zhang Y, Wan Z, He W, Zhang S, Yang L, Tan Q, Li W, Zhang Q, Gong F, Lu G, Tan YQ, Lin G, Du J. Extended application of PGT-M strategies for small pathogenic CNVs. *J Assist Reprod Genet*. 2024. <https://doi.org/10.1007/s10815-024-03028-6>.
- Giuliano R, Maione A, Vallefucio A, Sorrentino U, Zuccarello D. Preimplantation genetic testing for genetic diseases: limits and review of current literature. *Genes (Basel)*. 2023;14(11):2095. <https://doi.org/10.3390/genes14112095>.
- Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, Nagy ZP, Ubaldi FM. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod*. 2014;29(6):1173–81. <https://doi.org/10.1093/humrep/deu033>.
- Cimadomo D, Soscia D, Vaiarelli A, Maggiulli R, Capalbo A, Ubaldi FM, Rienzi L. Looking past the appearance: a comprehensive description of the clinical contribution of poor-quality blastocysts to increase live birth rates during cycles with aneuploidy testing. *Hum Reprod*. 2019;34(7):1206–14. <https://doi.org/10.1093/humrep/dez078>.
- Cornelisse S, Zagers M, Kostova E, Fleischer K, van Wely M, Mastenbroek S. Preimplantation genetic testing for aneuploidies (abnormal number of chromosomes) in in vitro fertilisation. *Cochrane Database Syst Rev*. 2020;9(9):CD005291. <https://doi.org/10.1002/14651858.CD005291.pub3>.
- Ma S, Liao J, Zhang S, Yang X, Hoher B, Tan J, Tan Y, Hu L, Gong F, Xie P, Lin G. Exploring the efficacy and beneficial population of preimplantation genetic testing for aneuploidy start from the oocyte retrieval cycle: a real-world study. *J Transl Med*. 2023;21(1):779. <https://doi.org/10.1186/s12967-023-04641-2>.
- Capalbo A, Rienzi L. Mosaicism between trophectoderm and inner cell mass. *Fertil Steril*. 2017;107(5):1098–106. <https://doi.org/10.1016/j.fertnstert.2017.03.023>.
- Riesterberg CK, Mok T, Ong JR, Platt LD, Han CS, Quinn MM. Sonographic abnormalities in pregnancies conceived following IVF with and without preimplantation genetic testing for aneuploidy (PGT-A). *J Assist Reprod Genet*. 2021;38(4):865–71. <https://doi.org/10.1007/s10815-021-02069-5>.
- Alteri A, Cermisoni GC, Pozzoni M, Gaeta G, Cavoretto PI, Viganò P. Obstetric, neonatal, and child health outcomes following embryo biopsy for preimplantation genetic testing. *Hum Reprod Update*. 2023;29(3):291–306. <https://doi.org/10.1093/humupd/dmad001>.
- Mao D, Xu J, Sun L. Impact of trophectoderm biopsy for preimplantation genetic testing on obstetric and neonatal outcomes: a meta-analysis. *Am J Obstet Gynecol*. 2024;230(2):199–212.e5. <https://doi.org/10.1016/j.ajog.2023.08.010>.
- Bunnell ME, Dobson LJ, Lanes A, Ginsburg ES. Use of preimplantation genetic testing for monogenic disorders and subsequent prenatal care and diagnostic testing. *Prenat Diagn*. 2022;42(8):1022–30. <https://doi.org/10.1002/pd.6189>.

26. Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tümer Z, Monk D, Mackay DJ, Grønsvov K, Riccio A, Linglart A, Netchine I. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenetics*. 2015;7:123. <https://doi.org/10.1186/s13148-015-0143-8>.
27. Mateu-Brull E, Rodrigo L, Peinado V, Mercader A, Campos-Galindo I, Bronet F, García-Herrero S, Florensa M, Milán M, Rubio C. Interchromosomal effect in carriers of translocations and inversions assessed by preimplantation genetic testing for structural rearrangements (PGT-SR). *J Assist Reprod Genet*. 2019;36(12):2547–55. <https://doi.org/10.1007/s10815-019-01593-9>.
28. Fan J, Zhang X, Chen Y, Zhang J, Zhang L, Bi X, Wang J, Huang X, Yan M, Wu X. Exploration of the interchromosomal effects in preimplantation genetic testing for structural rearrangements based on next-generation sequencing. *Mol Genet Genomic Med*. 2022;10(9):e2017. <https://doi.org/10.1002/mgg3.2017>.
29. Klimczak AM, Reig A, Neal SA, Seli E, Scott RT Jr. Interpretation of noninvasive prenatal testing results following in vitro fertilization and preimplantation genetic testing for aneuploidy. *Am J Obstet Gynecol MFM*. 2020;2(4):100232. <https://doi.org/10.1016/j.ajogmf.2020.100232>.
30. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol*. 2004;191(1):45–67. <https://doi.org/10.1016/j.ajog.2004.03.090>.
31. Grati FR, Malvestiti F, Ferreira JC, Bajaj K, Gaetani E, Agrati C, Grimi B, Dulcetti F, Ruggeri AM, De Toffol S, Maggi F, Wapner R, Gross S, Simoni G. Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. *Genet Med*. 2014;16(8):620–4. <https://doi.org/10.1038/gim.2014.3>.
32. Srebniak MI, Joosten M, Knapen MFCM, Arends LR, Polak M, van Veen S, Go ATJI, Van Opstal D. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2018;51(4):445–52. <https://doi.org/10.1002/uog.17533>.
33. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175–84. <https://doi.org/10.1056/NEJMoa1203382>.
34. Greco E, Biricik A, Cotarello RP, Iammarone E, Rubino P, Tesarik J, Fiorentino F, Minasi MG. Successful implantation and live birth of a healthy boy after triple biopsy and double vitrification of oocyte-embryo-blastocyst. *Springerplus*. 2015;14(4):22. <https://doi.org/10.1186/s40064-015-0788-y>.
35. Munné S, Blazek J, Large M, Martinez-Ortiz PA, Nisson H, Liu E, Tarozzi N, Borini A, Becker A, Zhang J, Maxwell S, Grifo J, Babariya D, Wells D, Fragouli E. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. *Fertil Steril*. 2017;108(1):62–71.e8. <https://doi.org/10.1016/j.fertnstert.2017.05.002>.
36. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, Wells D. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. *Hum Genet*. 2017;136(7):805–19. <https://doi.org/10.1007/s00439-017-1797-4>.
37. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, Cimadomo D, Benini F, Farcomeni A, Cuzzi J, Rubio C, Albani E, Sacchi L, Vaiarelli A, Figliuzzi M, Findikli N, Coban O, Boynukalin FK, Vogel I, Hoffmann E, Livi C, Levi-Setti PE, Ubaldi FM, Simón C. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. *Am J Hum Genet*. 2021;108(12):2238–47. <https://doi.org/10.1016/j.ajhg.2021.11.002>.
38. Abhari S, Kawwass JF. Pregnancy and neonatal outcomes after transfer of mosaic embryos: a review. *J Clin Med*. 2021;10(7):1369. <https://doi.org/10.3390/jcm10071369>.
39. Capalbo A, Poli M, Jalas C, Forman EJ, Treff NR. On the reproductive capabilities of aneuploid human preimplantation embryos. *Am J Hum Genet*. 2022;109(9):1572–81. <https://doi.org/10.1016/j.ajhg.2022.07.009>.
40. Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, Baldi M, Cursio E, Minasi MG, Greco E. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. *Fertil Steril*. 2018;109(1):77–83. <https://doi.org/10.1016/j.fertnstert.2017.09.025>.
41. Viotti M, Greco E, Grifo JA, Madjunkov M, Librach C, Cetinkaya M, Kahraman S, Yakovlev P, Kornilov N, Corti L, Biricik A, Cheng EH, Su CY, Lee MS, Bonifacio MD, Cooper AR, Griffin DK, Tran DY, Kaur P, Barnes FL, Zouves CG, Victor AR, Besser AG, Madjunkova S, Spinella F. Chromosomal, gestational, and neonatal outcomes of embryos classified as a mosaic by preimplantation genetic testing for aneuploidy. *Fertil Steril*. 2023;120(5):957–66. <https://doi.org/10.1016/j.fertnstert.2023.07.022>.
42. ESHRE Working Group on Chromosomal Mosaicism; De Rycke M, Capalbo A, Coonen E, Coticchio G, Fiorentino F, Goossens V, Mcheik S, Rubio C, Sermon K, Sfontouris I, Spits C, Vermeesch JR, Vermeulen N, Wells D, Zambelli F, Kakourou G. ESHRE survey results and good practice recommendations on managing chromosomal mosaicism. *Hum Reprod Open*. 2022;2022(4):hoac044. <https://doi.org/10.1093/hropen/hoac044>.
43. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, Cheng EH, Lee MS, Horcajadas JA, Corti L, Fiorentino F, Spinella F, Minasi MG, Greco E, Munné S. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. *Fertil Steril*. 2021;115(5):1212–24. <https://doi.org/10.1016/j.fertnstert.2020.11.041>.
44. Grati FR, Gallazzi G, Branca L, Maggi F, Simoni G, Yaron Y. An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening. *Reprod Biomed Online*. 2018;36(4):442–9. <https://doi.org/10.1016/j.rbmo.2018.01.005>.
45. Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroselli S, Coban O, Findikli N, Boynukalin FK, Bahceci M, Chopra R, Canipari R, Cimadomo D, Rienzi L, Ubaldi F, Hoffmann E, Rubio C, Simon C, Capalbo A. Incidence, origin, and predictive model for the detection and clinical management of segmental aneuploidies in human embryos. *Am J Hum Genet*. 2020;106(4):525–34. <https://doi.org/10.1016/j.ajhg.2020.03.005>.
46. Picchetta L, Ottolini CS, Neill HC, Capalbo A. Investigating the significance of segmental aneuploidy findings in preimplantation embryos. *F S Sci*. 2023;4(2):17–26. <https://doi.org/10.1016/j.xfss.2023.03.004>.
47. Documento di Buona Pratica per la Diagnosi Citogenetica e Citogenomica Costituzionale. https://sigu.net/wp-content/uploads/2023/09/BuonaPraticaCitogeneticaCitogenomica_v2022_REV.pdf.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.